Brain Pathology 11: 408-413(2001)

RESEARCH ARTICLE

Prevalence of HSV-1 LAT in Human Trigeminal, Geniculate, and Vestibular Ganglia and Its Implication for Cranial Nerve Syndromes

Diethilde Theil[1], Viktor Arbusow [1], Tobias Derfuss[2], Michael Strupp[1], Matthias Pfeiffer[3], Andrea Mascolo[4], Thomas Brandt[1]

[1] Department of Neurology, Klinikum Grosshadern, LMU Munich, Germany
[2] Max Planck Institute of Neurobiology, Martinsried, Germany
[3] Thermo Hybaid GmbH, Ulm, Germany
[4] Department of Neurology, University of Pavia, Italy

Herpes simplex virus type 1 (HSV-1) enters sensory neurons and can remain latent there until reactivation. During latency restricted HSV-1 gene expression takes place in the form of latency-associated transcripts (LAT). LAT has been demonstrated to be important not only for latency but also for reactivation, which may cause cranial nerve disorders.

Tissue sections of the trigeminal ganglia (TG), geniculate ganglia (GG), and the vestibular ganglia (VG) from seven subjects were examined for the presence of LAT using the *in situ* hybridization technique. LAT was found on both sides in all TG (100%), on both sides of five subjects (70%) in the GG, and in none of the VG. Using a second more sensitive detection method (RT-PCR), we found LAT in the VG of seven of ten other persons (70%).

This is the first study to demonstrate viral latency in the VG, a finding that supports the hypothesis that vestibular neuritis is caused by HSV-1 reactivation. The distribution of LAT in the cranial nerve ganglia indicates that primary infection occurs in the TG and GG and subsequently spreads along the facio-vestibular anastomosis to the VG.

Introduction

Herpes virus simplex type 1 (HSV-1) has been linked to a variety of clinical syndromes of the nervous system. After primary infection of the epithelium (stomatitis aphtosa), HSV-1 enters the axon terminals and is carried by axonal transport to sensory neurons of the human trigeminal ganglia (TG), where it can become latent. Demonstration of viral DNA does not prove the latency of HSV-1; it is necessary to ascertain viral transcription of the latency associated transcript (LAT), which accumulates in the nuclei of sensory neurons (30). Certain stimuli can reactivate HSV-1 by switching its viral state from latent to lytic. This change is reflected by the transcription and expression of all viral genes.

The *in situ* hybridization technique has been used to demonstrate LAT in neurons of the human TG (9, 10) and the geniculate ganglia (GG) (18). An attempt to demonstrate LAT in vestibular ganglia (VG) proved unsuccessful (17). No other studies have focused on this issue, although genomic HSV-1 DNA has consistently been detected in the VG of a high percentage of humans (2, 17, 29).

The major aims of the present study were to prove whether LAT can be determined in the VG by using a more sensitive method and whether the distribution of LAT differs in the three cranial nerve ganglia on both sides of a single person.

An affirmative answer to the first question would support the hypothesis that vestibular neuritis, the third most common cause of peripheral vestibular vertigo, is due to reactivation of HSV-1 in the VG. An answer to the second question would elucidate whether viral spreading occurs from the GG to the VG or along a preformed anatomical anastomosis. Both answers would be of clinical significance.

Material and Methods

The Ethics Committee of the Medical Faculty of the Ludwig-Maximilians University of Munich approved the use of autopsy samples for the present study.

TG, GG, and VG of both sides were removed from seven subjects (19 to 80 years old) 6 to 24 h after death. In a second experiment RT-PCR was performed on only the VG from ten other persons (aged 22 to 58). The

Corresponding author:
Dr. Diethilde Theil, Klinikum Grosshadern, Neurologisches Forschungshaus, Marchionistrasse 23, 81371 Munich, Germany; Tel: +49-89-70954816; Fax: +49-89-70954805; E-mail: dtheil@brain.nefo.med.uni-muenchen.de

cause of death of the subjects in the study was related to trauma; none of them had lesions suggestive of an active orolabial herpes infection. They also did not have a history of having had cranial nerve disorders.

The *in situ* hybridization technique and the reverse transcription polymerase chain reaction (RT-PCR) were used concurrently to detect viral RNA, which indicates latency in the VG.

For *in situ* hybridization the ganglia were frozen immediately in Tissue tek® compound (Sakura, Zoeterwoude, The Netherlands) on dry ice. The 10-μm sections were cut and mounted on positively charged slides (SuperFrost*/Plus®, Menzel, Braunschweig) and stored at -20°C until use.

A synthetic gene (HSV-1 nucleotide positions 119,195 to 120,029) assembled using HPLC- and PAGE-purification oligonucleotides and a 285-bp PCR product (HSV-1 nucleotide position 120704 to 120898) were individually cloned into the pGEM-T Easy plasmid (Promega, Mannheim) containing the SP6 and T7 bacteriophage RNA polymerase promoters. A 347-bp fragment of a stable LAT intron (HSV-1 nucleotide positions 119,628 to 119,978) cloned into plasmid ATD19 was kindly provided by T. Margolis (15). The 800 bp RNA probe (P15) is complementary to the 5′ end of the LAT transcript and stretches the stable intron to the potential LAT ORF1, whereas the 285 bp RNA probe (P12) is complementary to the 3′ end of the LAT transcript and spans the ORF2 and part of the ORF-ICPO (32). The third RNA probe used (ATD19) stretched a 347bp fragment complementary to the stable intron of LAT.

From the above described fragments RNA probes were prepared by *in vitro* transcription using the DIG RNA labeling kit (SP6/T7) (Roche Molecular Biochemicals, Mannheim). The appropriate RNA polymerase (SP6, T7 and T3 in case of ATD19) was added to the reaction mixture containing digoxigenin-labeled uridine triphosphate (DIG-11-UTP). Single-strand RNA probes were synthesized from either strand of the DNA template according to the manufacturer's instructions. The antisense RNA probes were capable of hybridizing the LAT transcripts, whereas sense RNA probes were used as negative controls.

In situ hybridization. Tissue sections were thawed and dried for 15 min at 50°C. The protocol used was largely derived from those of Cunningham and DeSouza (11). Briefly, sections were fixed in 4% paraformaldehyde for 20 min, proteinase-K digested (10 μg/ml) for 20 min, postfixed in 4% paraformaldehyde for 5 min, and acetylated with 0.25% in 0.1 M triethanolamine.

Sections were washed in 2×SSC and then prehybridized at 45°C for 1 h in prehybridization buffer (50% formamide, 0.3M NaCl, 10mM Tris-HCL pH 8.0, 1mM EDTA pH 8.0, tRNA 0.5mg/ml, dextran sulphate 100 mg/ml, 1× Denhardt's solution).

RNA probes (10 ng/ml) were added to prehybridization solution, applied to tissue sections, and allowed to incubate at 45°C for 16 h. After hybridization, sections were washed in 2×SSC and then treated with 2 μg/ml RNase A (Sigma, Germany). Two washing steps were carried out sequentially at 55°C in 2×SSC and 0.5×SSC. The hybridized probes were detected using alkaline phosphate conjugated anti-digoxigenin antibodies included in the DIG Nucleic Acid Detection Kit (Roche Molecular Biochemicals, Mannheim).

RT-PCR. VG of ten subjects were collected in RNAlater™ solution (ams Biotechnology, Abington, UK). Left and right VGs of each subject were pooled and homogenized with Trizol® (Life Technologies, Karlsruhe). RNA was extracted according to the manufacturer's instructions.

To ensure integrity of RNA, we performed standard cDNA synthesis and subsequent PCR with primers specific for beta actin cDNA (b-actin1: 5′-CCTCGCCTTTGCCGATCC; b-actin2: 5′-GGATCTTCATGAGGTAGTCAGTC;) (27). First-strand cDNA was synthesized from 5 μg of total VG RNA using oligo-d$(T)_{12}$, random hexamers (Roche Molecular Biochemicals, Mannheim), and Moloney murine leukemia virus reverse transcriptase (M-MLV) (Promega, Mannheim). The cDNA of all VG was amplified with primers (*ICP27a* 5′-CGCCAAGAAAATTTCATCGAG; *ICP27b* 5′-ACATCTTGCACCACGCCAG [8]) specific for immediate-early (IE) gene transcript ICP27. As a positive control cDNA from Vero cells infected with HSV-1 was used.

Since the major species of LAT are not polyadenylated (16), 5 μg of total VG RNA were hybridized to 0.5 μg of LAT downstream primer in a separate reaction in order to detect LAT. First strand synthesis for all reactions was carried out at 42°C as specified by the supplier. Tenfold serial dilution of 1/10 of the RT products synthesized with the LAT primer were amplified with 0.5 μg of each of the LAT primers (LATa 5′-ACGAGGGAAAACAATAAGGG; LATb 5′-GACAGCAAAAATCCCCTGAG [21]). In addition, 1 μg of RNA of each sample was amplified directly with the LAT primer pairs without RT to ensure that the signal detected by RT-PCR was not due to DNA contamination

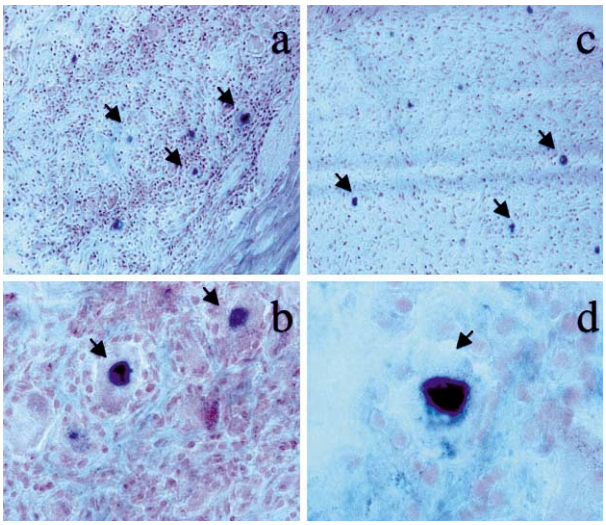

Figure 1. *In situ* hybridization using the ATD19 probe in the TG (ab) and GG (cd). Clusters of positive neurons (*arrows*) were found in the TG (**A**) (original magnification ×200) and GG (**C**) (original magnification ×100). The positive hybridization signal is confined to only the nuclei of ganglionic neurons in the TG (**B**) (×400) and GG (**D**) (oil immersion)

of the RNA sample. As a further control for RNA purity, selected RNA samples were also DNase treated (20 U/5μg RNA) (DNase I, Roche Biochemicals, Mannheim).

RNA from a TG positive by *in situ* hybridization was used to establish the LAT RT-PCR. The PCR was performed with 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min (for LAT primers), at 60°C for 1 min (for the ICP27 and beta-actin primer), and extending at 72°C for 2 min.

PCR products were separated in 1.5% (for LAT) and 2.5% (for ICP27) agarose gels.

Results

In situ hybridization. A positive hybridization signal for LAT was detected in all TG, in most GG, but in none of the VG. LAT was always found bilaterally. No difference in the spread of LAT was apparent in the right and left ganglia of the same person. Positive cells occurred in small clusters throughout multiple sections of the same ganglion of the TG (Figure 1 a, b), whereas single discrete positive cells were a common finding in the GG. Small clusters of positive cells were found in the GG of only three subjects (Figure 1 c, d). The signals were less abundant when the P12 probe was used rather than the ATD19 and P15 probes, but the pattern of the hybridization signal remained the same for all three probes.

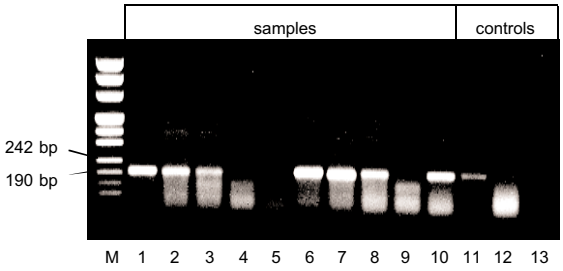

Figure 2. LAT RT-PCR results for the VG. Lanes 1 to 10 represent cDNA samples from the VG of ten subjects. A 195 bp product was obtained in seven of ten samples. Lane 11 shows the positive control (cDNA from a TG positive for LAT by *in situ* hybridization). Lane 12 shows the negative control (cDNA from muscle tissue) and lane 13, the water control. M represents the pUC mix maker, 8 (MBI Fermentas, Germany).

LAT RT-PCR. The *in situ* hybridization in the bilateral VG of seven subjects was negative. To detect the accumulation of LAT at a higher level of sensitivity, we applied the RT-PCR technique to the VG of ten other subjects. The expected 195 bp band was present in seven of ten tested pairs of vestibular ganglia (Figure 2). The experiments were run twice with the respective controls. RNA of the corresponding samples without reverse transcription was amplified with the LAT primers and was found to be negative (data not shown). Samples treated before the RT reaction with RNase free DNase I gave the same results as without the DNase treatment. Furthermore, RNA from muscle tissue was transcribed and amplified with the LAT primers. No amplifiable product was found in the muscle sample (Figure 2).

ICP27 RT-PCR. A definitive confirmation that the virus is in a latent state is the absence of lytic viral transcripts. Products of the immediate-early genes are essential for transcriptional activation of early and late genes and ultimately for virus growth and the lytic cycle. The ICP27 transcript plays a key role in initiating the productive cycle (23). No amplifiable ICP27 transcript could be detected in the poly-A RNA fraction of the VG from ten subjects. As expected, no amplifiable product was detected in the negative control (muscle sample). The 63bp ICP27 product was detected only in the cDNA samples of Vero cell infected with the HSV-1 strain (Figure 3).

Discussion

To determine whether reactivation of the HSV-1 is indeed implicated in the etiology of cranial nerve disorders, the transcriptional activity of the virus needs to be

established *in vivo*. Demonstration of viral LAT indicates that the virus is in a latent state and viral genomes are available to reactivate a productive infection (32). Thus far, reactivation of HSV-1 from the TG has been shown to cause recurrent herpes labialis (3, 4, 9, 10). There is also compelling evidence that reactivation from the GG causes idiopathic peripheral facial palsy (Bell's palsy) (1, 18, 24, 26).

Latent HSV-1 in the VG. In the present study we demonstrated for the first time the presence of LAT in the VG. Since we did not detect other viral transcripts essential for the lytic state in these VG, the HSV-1 was almost certainly in a latent state. This finding strengthens the hypothesis that vestibular neuritis is caused by a reactivation of latent HSV-1 from the VG. Until now this hypothesis was based only on temporal bone studies, which revealed that changes in the vestibular nerve were similar to those usually seen in viral infections (25, 28), and on studies that detected genomic HSV-1 DNA in the VG (2, 18). Additional support derives from animal studies that demonstrated HSV-1 antigen in the VG and vestibular nerve of mice experimentally infected with HSV-1 (12, 19) and an electron microscopy study that found virus-like structures in the human VG (20).

Using the *in situ* hybridization technique, we were not able to detect LAT in the VG, but we did readily detect LAT by RT-PCR in the non-poly (A) RNA fraction. The low levels of RNA are not of importance for reactivation, since experimental studies have shown that reactivation can occur in infected neurons that express very low levels of LAT (13). A low accumulation of LAT suggests the presence of a distinctive mechanism that regulates and processes LAT in the neurons of the VG. This agrees with findings of recent studies that LAT synthesis differs in certain neurons depending on the host transcription factors (7, 33). Moreover, a smaller amount of LAT and a different processing method could influence the pathophysiology and clinical manifestation of a reactivation in the VG. In this connection it is interesting that inflammation in Bell's palsy can be regularly demonstrated by gadolinium–diethylenentriamine enhancement, whereas in another study (31) contrast enhancement could not be demonstrated in 60 patients with vestibular neuritis.

Distribution of latent HSV-1 in the cranial nerve ganglia. The second aim of our study was to determine how LAT is distributed in the cranial nerve ganglia. First, in contrast to previous studies (10, 17, 18) we found LAT in far more ganglia (100% in TG and 70% in GG by *in situ* hybridization and 70% in VG by RT-PCR). Again, this higher number of positive ganglia can be explained by the use of frozen tissue samples, since RNA is better preserved in frozen tissue than in paraffin-embedded tissue. Second, the tested subjects showed bilateral infection of the TG and GG. Due to the small amount of RNA in the VG, the RNA was pooled for each individual for RT-PCR. For this reason we cannot draw any conclusions about the side of distribution of LAT in the VG.

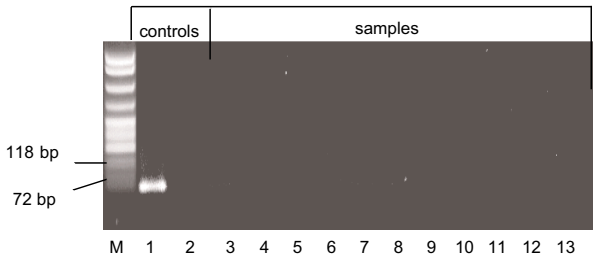

Figure 3. ICP27 RT-PCR results for the VG. Lane 1 shows the positive control (cDNA from Vero cells infected with HSV-1 wild type). Lane 2 shows the negative control (cDNA from muscle tissue). Lanes 3 to 12 represent cDNA samples of the VG of the ten subjects tested for LAT, and lane 13 shows the water control. The 63bp ICP27bp product was detectable only in the positive control (lane 1). M represents the $\phi\times174$ DNA-Hae III Digest marker (New England BioLabs, Germany).

A recent study (8) also reported that HSV-1 infects both sides of the TG equally. Using real time PCR, the authors found that the number of HSV-1 genomes did not significantly differ between the left and right TG of the same individual. However, they found that LAT expression on the left and right sides varied greatly. Although the techniques used in our study were not suitable for quantifying LAT, we assume that the relative expression of LAT also varied in our subjects.

A variation in the level of LAT expression in the left and right cranial nerve ganglia may account for unilateral reactivation of HSV-1, since HSV-1 would reactivate more rapidly from the side with greater LAT expression. Reactivation could still occur in the counterpart ganglion, but no damage or clinical disease would be apparent because of the immune response already mounted. However, data from experimental animal studies on the quantity of LAT and reactivation frequency are still controversial (6, 14, 22).

The LAT probes used for *in situ* hybridization mapped to three distinctive regions of the LAT gene which span three putative open reading frames. Since the pattern of the hybridization signal stayed the same with all LAT probes, we assume that there is no change

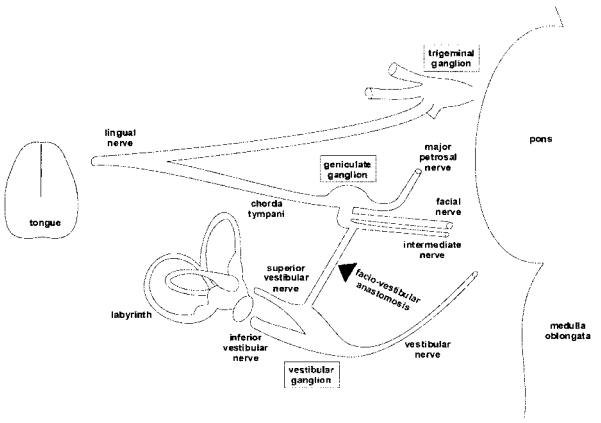

Figure 4. Schematic drawing of the anatomic connections between the TG, GG, and VG. Viral spreading is possible via the lingual nerve to the GG and via the corda tympani to the GG, and then to the VG via the facio-vestibular anastomosis (*arrow*).

in the splicing or transport activities of the LAT in the neurons of the TG and GG.

Our finding that the signals of LAT decreased from the TG (highest) to GG and VG (lowest) is compatible with the hypothesis that HSV-1 migrates along the lingual nerve to the TG and GG, where it becomes quiescent (see Figure 4). When reactivating from these sites, HSV-1 could migrate in two different directions: *1)* along the lingual nerve back to the oral mucosa and/or *2)* along the the facio-vestibular anastomosis to the VG (2, 5). HSV-1 might also spread concomitantly to all cranial ganglia, and the differences in the LAT signals could be due to the different number of susceptible neurons in these ganglia. To obtain more information on viral spreading, *in situ* PCR or *in situ* RT-PCR needs to be applied for viral detection in the VG. Moreover, a combination of this technique and immunohistochemical staining, which can detect the neuron types that harbor latent viruses, could reveal more information about the predilection of the latent virus for certain neurons and indirectly about the host cell factors involved in the regulation of viral gene expression. A recent study (33) showed that only a few neuronal phenotypes permit the establishment of latent infection. Thus, a better characterization of the anatomical site of infection could produce evidence that certain neurological syndromes mentioned in this study are caused by the reactivation of HSV-1 from the respective ganglia.

Acknowledgements

We are grateful to Dr. D. Gilden for providing the positive TG ganglia. Dr. Gilden sent us a TG that was positive by LAT PCR in order to help us establish the *in situ* hybridization technique. The authors also thank Dr. T. Margolis for providing the ATD 19 plasmid and Dr. H. Fickenscher for providing the HSV-1 infected Vero cells. We are indebted to Ms. Judy Benson for carefully reading and editing the manuscript.

References

1. Adour KK, Bell DN, Hilsinger RL (1975) Herpes simplex virus in idiopathic facial paralysis (Bell palsy). *JAMA* 233: 527-530
2. Arbusow V, Schulz P, Strupp M, Dietrich M, von Reinhardstoettner A, Rauch E, Brandt T (1999) Distribution of herpes simplex virus type 1 in human geniculate and vestibular ganglia: implications for vestibular neuritis. *Ann Neurol* 46: 416-419
3. Baringer JR, Swoveland P (1973) Recovery of herpes-simplex virus from human trigeminal ganglions. *N Engl J Med* 288: 648-650
4. Bastian FO, Rabson AS, Yee CL, Tralka TS (1972) Herpesvirus hominis: isolation from human trigeminal ganglion. *Science* 178: 306-307
5. Bergström B (1973) Morphology of the vestibular nerve. II. The number of myelinated vestibular nerve fibers in man at various ages. *Acta Otolaryngol. (Stockh)* 76:173-179
6. Bourne N, Stanberry LR, Connelly BL, Kurawadwala J, Straus SE, Krause PR (1994) Quantity of latency-associated transcript produced by herpes simplex virus is not predictive of the frequency of experimental recurrent genital herpes. *J Infect Dis* 169: 1084-1087
7. Chen X, Schmidt MC, Goins WF, Glorioso JC (1995) Two herpes simplex virus type 1 latency-active promoters differ in their contributions to latency-associated transcript expression during lytic and latent infections. *J Virol* 69: 7899-7908
8. Cohrs RJ, Randall J, Smith J, Gilden DH, Dabrowski C, van der Keyl H, Tal-Singer R (2000) Analysis of individual human trigeminal ganglia for latent herpes simplex virus type 1 and varicella-zoster virus nucleic acids using real-time PCR. *J Virol* 74: 11646-11471
9. Corey L, Spear PG (1986) Infections with herpes simplex viruses (2). *N Eng J Med* 314: 686-691, 749-757
10. Croen DK, Ostrove JM, Dragovic LJ, Smialek JE, Straus SE (1987) Latent herpes simplex virus in human trigeminal ganglia. Detection of an immediate early gene 'antisense' transcript by *in situ* hybridisation. *N Engl J Med* 317: 1427-1432
11. Cunningham ET, DeSouza EB (1994) Localization of type I IL-1 receptor mRNA in the brain and endocrine tissues using in situ hybridization histochemistry. In: *Methods in Neuroscience. Neurobiology of Cytokines*, DeSouza EB (ed); Part A, pp. 112-127. CRC Press, New York
12. Davis LE (1993) Viruses and vestibular neuritis: review of human and animal studies. *Acta Otolaryngo Suppl* 503: 70-73

13. Ecob-Prince M, Hassan K (1994) Reactivation of latent herpes simplex virus from explanted dorsal root ganglia. *J Gen Virol* 75: 2017-2028
14. Ecob-Prince M, Rixon FJ, Preston CM, Hassan K, Kennedy PGE (1993) Reactivation in vivo and in vitro of herpes simplex virus from mouse dorsal root ganglia which contain different levels of latency-associated transcripts. *J Gen Virol* 74: 995-1002
15. Ellison AR, Yang L, Voytek C, Margolis TP (2000) Establishment of latent herpes simplex virus type 1 infection in resistant, sensitive, and immunodeficient mouse strains. *Virology* 268: 17-28
16. Farrell MJ, Dobson AT, Feldman LT (1991) Herpes simplex virus latency-associated transcript is a stable intron. *Proc Natl Acad Sci USA* 88: 790-794
17. Furuta Y, Takasu T, Fukuda S, Ynuyama Y, Sato KC, Nagashima K (1993) Latent herpes simplex virus type 1 in human vestibular ganglia. *Acta Otolaryngol Suppl* 503: 85-89
18. Furuta Y, Takasu T, Sato KC, Fukuda S, Inuyama Y, Nagashima K (1992) Latent herpes simplex virus type 1 in human geniculate ganglia. *Acta Neuropathol (Berl)* 84: 39-44
19. Hirata Y, Gyo K, Yanagihara N (1995) Herpetic vestibular neuritis: an experimental study. *Acta Otolaryngol Suppl* 519: 93-96
20. Kitamura K, Toriyama M (1985) Virus-like particles in human vestibular ganglion cells. *Arch Otorhinolaryngol* 241: 303-75
21. Lynas C, Cook SD, Laycock KA, Bradfield JW, Maitland NJ (1989) Detection of latent virus mRNA in tissues using the polymerase chain reaction. *J Pathol* 157: 285-289
22. Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM (1996) Correlation between herpes simplex virus type 1 rate of reactivation from latent infection and the number of infected neurons in trigeminal ganglia. *Virology* 225: 72-81
23. McCarthy A, McMahan L, Schaffer PA (1989) Herpes simplex virus type 1 ICP27 deletion mutants exhibit altered patterns of transcription and are DNA deficient. *J Virol* 63: 18-27
24. McCormick DP (1972) Herpes simplex virus as cause of Bell's palsy. *Lancet* I: 937-939
25. Morgenstein KM, Seung KI (1971) Vestibular neuronitis. *Laryngoscope* 81: 131-139
26. Murakami S, Mizobuchi M, Nakashiro Y, Doi T, Hato N, Yanagihara N (1996) Bell's palsy and herpes simplex virus: identification of viral DNA in endoneurial fluid and muscle. *Ann Intern Med* 124: 27-30
27. Raff T, van der Giet M, Endemann D, Wiederholt T, Paul M (1997) Design and testing of beta-actin primers for RT-PCR that do not co-amplify processed pseudogenes. *Biotechniques* 23: 456-460
28. Schuknecht HF, Kitamura K (1981) Second Louis H. Clerf Lecture. Vestibular neuritis. *Ann Otol Rhinol Laryngol Suppl* 90: 1-19
29. Schulz P, Arbusow V, Strupp, Dieterich M, Rauch E, Brandt T (1998) Highly variable distribution of HSV-1-specific DNA in human geniculate, vestibular, and spiral ganglia. *Neurosci Lett* 252: 139-142
30. Stevens JG, Wagner EK, Devi-Rao GB, Cook ML, Feldmann LT (1987) RNA complementary to a herpesvirus α gene mRNA is prominent in latently infected neurons. *Science* 235: 1056-9
31. Strupp M, Jager L, Muller-Lisse U, Arbusow V, Reiser M, Brandt T (1998) High resolution Gd-DTPA MR imaging of the inner ear in 60 patients with idiopathic vestibular neuritis: no evidence for contrast enhancement of the labyrinth or vestibular nerve. *J Vestib Res* 8: 427-433
32. Wagner EK, Devi-Rao G, Feldman LT, Dobson AT, Zhang YF, Flangan WM, Stevens JG (1988) Physical characterization of the herpes simplex virus latency-associated transcript in the neurons. *J Virol* 62: 1194-1202
33. Yang L, Voytek C, Margolis TP (2000) Immunohistochemical analysis of primary sensory neurons latently infected with herpes simplex virus type 1. *J Virol* 74: 209-217